# Are the Mediterranean forests in Southern Europe threatened from ozone?

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### Abstract

The influence of air pollutants on ecosystems in Europe has been studied for over two decades in the Western and Nordic countries and in the Alps. The impacts of air pollutants on Mediterranean forest ecosystems (evergreen sclerophyllous forests and maquis) are poorly understood. The Mediterranean climate encourages the generation of high concentrations of ozone - now recognised to be the most prevalent and damaging air pollutant to which vegetation is exposed in many regions. In this paper, we examine the way in which many of the typical morphological and ecophysiological features of Mediterranean vegetation influence ozone impacts, plus the way in which the combination of environmental stresses to which Mediterranean vegetation is exposed in the field affect responses to ozone. Sclerophyllous Mediterranean species (typified by leaves with dense mesophyll, little intercellular air space and containing an abundance of primary, e.g. ascorbate, and secondary metabolites, e.g. tannins and phenylpropanoids, that are capable of protecting key biomolecules from oxidative stress) might be expected to be hardier than their counterparts more typical of the relatively mesic environments of Northern and Continental Europe. Moreover, soil water shortage during the height of summer causes partial stomatal closure for lengthy periods each day. As a result, vegetation may avoid taking-up the ozone when concentrations are at their highest. There are several confirmed reports of visible symptoms of ozone damage (chlorotic mottle, necrosis, reddening etc.) on important crops and forest trees. The significance of these observations is discussed, along with the way in which ongoing changes in the Mediterranean environment may affect the future impacts of rising ozone concentrations on vegetation.

## Introduction

Mediterranean ecosystems are considered intrinsically fragile (Naveh, 1995) because of the climate (discontinuous rain, with prolonged periods of soil and atmospheric drought in summer; high summer temperatures; intense solar radiation) and edaphic conditions (often thin soils of poor nutritional quality – particularly scarce in nitrogen and phosphorus) to which they are exposed in their natural environment. In addition, they have been subjected to such extensive anthropogenic pressures that their original features are often scarcely recognizable. As far as the impact of gaseous pollutants are concerned, one particularly damaging form of air pollution – the photochemical generation of oxidants (and in particular, ozone) - finds the most favourable environment for its development and its diffusion in Mediterranean climates (Butkovic *et al.*, 1990). The detrimental effects of this type of air pollution on natural and managed ecosystems are well documented in Europe (Skärbi *et al*, 1998) and North America (Chappelka & Samuelson, 1998). In the Mediterranean basin there have been several studies which sought to quantify effects on the yield of agricultural crops (Benton *et al.*, 2000), but the impacts of photochemical oxidant pollution on forests and other natural or semi-natural ecosystems in the Mediterranean remain largely unresearched (Bussotti & Ferretti, 1998). In this context, we review herein what is known about the impacts of ozone on Mediterranean plants and ecosystems and assess the potential risks posed by photochemical oxidants as well as the way in which specific features of Mediterranean plants, and the environment to which they have adapted, might be expected to influence the impacts of ozone.

### Ozone climatology in the Mediterranean region

In the Mediterranean basin, the persistence during summer months of the Azores anti-cyclone over Europe, and the resulting atmospheric stability, with high temperatures, low relative humidity and high levels of solar irradiation, favour a massive photochemical production of ozone in the lower troposphere, with hourly concentration peaks that frequently reach 150-220 ppb or more (Chaloulakou *et al.*, 1999).

In rural and suburban areas located downwind from precursor emission sources, ozone reaches higher concentrations than those recorded in urban areas. In urban zones the presence of considerable NO emissions favours a titration reaction, reducing concentration of O<sub>2</sub> produced. In rural areas, where there are no significant pollutant sources, NO emissions occur far less frequently and the advection of NO<sub>2</sub> from the emission areas feeds the photolysis cycle and ozone production increases as a consequence. Breezes activated by the sea-coast or mountain-valley thermobaric differences contribute then to a redistribution of ozone throughout the region, affecting even remote areas such as coastal and mountain rural zones. The transport of ozone and its precursors from the highly anthropized zones to remote forest sites by summer breezes has been highlighted by several different studies carried out in mountain areas south of the Alps and in the Italian plains along the Po river (Bacci et al., 1990; Gerosa et al., 1999), in the Mediterranean coastland and inland regions of Spain (Martín et al., 1991), Italy (Lorenzini et al., 1995), Greece (Güsten et al., 1988), and in the South-Eastern Mediterranean (Alper-Siman et al., 1997).

During the day, sea breezes can transport inland ozone formed in the urban and industrial coastal areas for up to 100 km and further (Millán *et al.*, 1991, 1996; Lalas *et al.*, 1983). The evening sea breezes push the masses of air offshore where the deposition rate is so slow that ozone accumulates and is transported back to the coast the following day, when the daytime sea breezes resume, thus setting in motion mechanisms of photo-oxidant recirculation (Fortezza et al., 1993; Alper-Siman et al., 1997). A similar mechanism occurs where orographic barriers are present and during the day, the ozone plume is pushed upwards by ascending breezes. The formation of nocturnal inversion layers hinders its deposition and this gives rise to accumulated masses of ozone-rich air at altitudes ranging from 500 m to 2000 m above ground (Schlager et al., 1992). When phenomena of subsidence occur or when catabatic, or descending, currents drag these masses vertically downwards, they may actually contact the ground and give rise to peak ozone exposures, usually in the evening. Multiple layers of ozone accumulation have also been observed above the inland in coastal areas (Georgiadias et al., 1994; Millán et al., 1996; Sanz & Millán, 1998). Ozone concentration is highest in spring and summer, and lowest in winter. Sudden increases of ozone concentration, however, can be observed in winter as well, when phenomena causing a vertical mixing of the atmosphere occur, due to strong descending winds (such as föhn/stau in mountain areas) or to intrusions of air from the lower stratosphere / free troposphere, which are not infrequent in spring (Davies & Schuepbach, 1994).

Mediterranean vegetation is a strong emission source of isoprenoid compounds (monoterpenes and isoprene) released into the atmosphere (Street *et al.*, 1997; Peñuelas & Lusià, 1999). These compounds play an important role in the plants' defense mechanisms against heat stress (Loreto *et al.*, 1998). Yet, these natural emissions are a considerable source of active carbon and can therefore play a crucial role in the formation and persistence of atmospheric pollutants and greenhouse gases such as carbon monoxide and the same ozone (Fehsenfeld *et al.*, 1992). The interaction of ozone with the crowns' emissions gives rise to other potentially toxic compounds such as carbonyl compounds and radicals (Fruekilde *et al.*, 1998).

Critical load for forests, AOT40 of 10,000 ppb.h (accumulated hourly exposures of  $O_3$  over a threshold of 40 ppb in daylight hours from April to September; Kärenlampi & Skärby, 1996) is vastly exceeded during the growing season in the Mediterranean and sub-alpine region, as is shown in the simulated experiments done using the EMEP model (Hjellbrekke, 2000) and by regional estimates prepared in individual countries (Posch *et al.*, 1998). Furthermore, in many sites across southern Europe the hourly concentration of  $O_3$  in summer never drops below the threshold of 40 ppb (Velissariou & Skretis, 1999). Table 1 shows some relevant data in Mediterranean sites.

# Ecological behaviour of Mediterranean woody plants in relation to ozone

The phytotoxic action of ozone depends on the way it is absorbed by the leaves and, therefore, how it spreads in the mesophyll. Mediterranean vegetation consists to a large extent of coriaceous leaf plants (evergreen sclerophyllous vegetation). The foliar structure of Mediterranean evergreens is usually characterized by the presence of 2-3 layers of palisade mesophyll and a thinner layer of spongy tissue (see De Lillis, 1991). This is a strategy which limits the transpiration, but in doing so it also limits the absorption of CO<sub>2</sub> and, therefore, of atmospheric pollutants. The typical Mediterranean conditions enhance the sclerophylly. In fact, plants growing in dry, sterile environments, and/or subjected to high radiation intensity usually have greater foliar thickness and density (Gutschick, 1999), thus suggesting an infra-specific sensitivity: ozone exerts a lesser impact on trees growing under conditions of stress (see also Davison & Barnes, 1998). In addition, at their southernmost distribution area, several species largely distributed in Europe (European beech: Bauer et al., 1997; silver fir: Rinallo & Gellini, 1989) differ from the central European provenances in that their leaves are more sclerophyllous. In the case of silver fir (Larsen, 1990) and European beech (Paludan-Müller et al., 1999) a reduced sensitivity to ozone and other air pollutants has also been observed in the southern provenances .

Stomatal activity is considered the key element determining the sensitivity of a particular species to ozone (Emberson *et al.*, 2000). Usually the prevailing weather conditions induce a marked reduction in stomatal conductance in Mediterranean vegetation during the height of summer. The highest levels of ozone experienced in the field usually coincide with the time that non-managed plants in the Mediterranean suffer the greatest degree of water deficit, and their stomata are close. However, the behaviour of individual species varies considerably (Rhizopoulou & Mitrakos, 1990; Tretiach, 1993; Gucci et al., 1999) in their capacity to tolerate drought before resorting to stomatal closure. As a consequence, those species that exhibit the greatest ability to maintain, or reactivate, gas exchange under conditions of water stress, might be expected to be the most affected by ozone. Generally speaking, the deciduous trees narrow their stomata to higher water potential than the evergreen ones. For example, in Quercus ilex leaves stomata remain open much longer than in deciduous oaks (Salleo & Lo Gullo, 1990; Acherar et al., 1991). Between the evergreen shrubs, Phillyrea latifolia L. shows the highest tolerance to water stress.

The way in which the combination of ozone and soil water deficit affect stomatal conductance are, however, much more complex than might first be perceived since recent evidence suggests that exposure to the pollutant can impair stomatal performance under conditions of soil water deficit (Maier-Maercker, 1998) and the outcomes in terms of cost/benefit analysis probably depend, at least to some extent, on the timing of the stress to which the plant is exposed, genotype and the intensity of the stress to which the plant is exposed (Maier-Maerker, 1998).

Biochemical processes leading to compensation (for the damaged tissues) and to protection (by means of the production of anti-oxidant substances) presuppose considerable metabolic costs (Heath, 1999), which therefore lead to an increase in carbohydrate

*Table 1.* Number of hours and days exceeding 200 µg m<sup>-3</sup>, maximum concentrations and AOT40 (April-September, daylight) in 1998 at selected EMEP monitoring sites (from Hjelbrekke, 2000 modified)

ppb.h
21469
21469
2.100
7968
20694
39934
24945
24630
21342
17427
6805
2 3 2 2 2 1

consumption, with carbohydrates being used for purposes other than plant growth. From this point of view, primary (ascorbate) and secondary metabolites (including tannins and other phenolic substances) play an important role (Polle & Rennenberg, 1992). Secondary metabolites are normally present in many plants growing in a Mediterranean climate (Karabourniotis *et al.*, 1993), and their concentrations increase under environmental stress (Gravano *et al.*, 2000b; Tattini *et al.*, 2000). Since the same substances are involved in detoxification from ozone stress processes (Kangasjärvi *et al.*, 1994), plants already endowed with these substances would appear to have a greater protection against this pollutant.

### Sensitivity and visible symptoms

In forest tree leaves visible symptoms attributable to chronic exposure to ozone were described for the first time in the mountain pines of a Mediterranean-type climate region, the Southwest of the United States (Miller et al., 1963, Miller & McBride, 1999). These symptoms consisted in localized yellowing (chlorotic mottles) which degenerated into necrotic patches, and only in the most severe cases did needle tip necrosis ensue. These yellowings were usually associated with a degeneration of the mesophyll (Evans & Miller, 1972). Among the woody species of Southern European flora and the Mediterranean basin, basal plane pines are the most sensitive to the action of photo-oxidants. Already in the early 1980s Naveh et al. (1980) described symptoms in Pinus pinea L. and Pinus halepensis Mill. in Israel. The most evident and widespread symptoms were later described in Pinus halepensis in Spain (Gimeno et al., 1992; Barnes et al., 1999), in Greece (Velissariou et al., 1992; Gimeno et al., 1992) and in Italy (Soda et al., 2000). Pinus halepensis needles develop a very typical symptomatology (chlorotic mottles), similar to that described by the above-mentioned American researchers. Sanz et al. (2000) found that the distribution pattern of ozone visual injuries (chlorotic mottle) in Pinus halepensis correlated with the penetration of the pollutant transported by the seabreeze into coastal valleys in Eastern Spain.

Broadleaved trees develop a varied symptomatology which has been described in several pictorial atlases and handbooks (see Skelly *et al.*, 1987; Flagler, 1998; Innes *et al.*, 2001). In Europe the most widespread and evident symptoms were reported in Southern Switzerland (Skelly *et al.*, 1998) and in adjacent areas in Northern Italy (Cozzi *et al.*, 2000; Gravano *et al.*, 2000a). These symptoms were validated by means of open top chamber experiments (VanderHeyden *et al.*, 2001). In the Mediterranean region the majority of available reports come from Spain (Skelly *et al.*, 1999; Sanz & Millán, 2000) where several evergreen shrubs were found to be sensitive. Table 2 gives an overall picture of the woody species in which symptoms were observed and the countries where the observations took place.

The sensitivity to ozone of Mediterranean species has also been investigated, by means of tests performed in controlled and/or semi-controlled environments, in terms of physiology and/or ultrastructure (Table 3). One can observe that the most widely studied species is Pinus halepensis, but it is difficult to extrapolate these findings to the field because almost all experiments were conducted with seedlings, whose response to ozone differs from that of adult trees (Kolb et al., 1998). In some cases, researchers have even used seedlings less than a year old, so that their foliar morphology is different from older trees. Further, the concentrations and exposure periods applied in these experiments are often highly unrealistic (concentrations greater than 100 ppb, although not rare, are normally only observed as hourly peak rates). Lastly, these experiments are performed in optimal soil moisture conditions, whereas Mediterranean species in summer are normally subjected to drought. Experiments only recently have been performed on typical evergreen broadleaved trees (Table 3). Among these, Arbutus unedo L. is potentially the most sensitive species.

The results of the investigations listed in Table 3 are largely specie-specific and are influenced by the experimental conditions. The most common results are the reduction of stomatal conductance and photosynthesis, as well as the content in chlorophyll. Detoxifying compounds (peroxidase, ascorbate) increase. The ozone effects in combination with water stress are controversial in experimental conditions.

#### **Discussion and Conclusions**

At first glance it would appear that Mediterranean forests are well endowed with a good resilience to ozone. The ecophysiological behaviour of trees and their foliar structure (well-balanced with the climatic and edaphic conditions of the environment), as well as the abundance of detoxifying metabolites in many species, allows them to limit the stomatal absorption of ozone and to repair any injury to their leaves very quickly. Evergreen Mediterranean plants are able to maintain a stomatal activity under moderate water stress, but display their strongest photosynthetic activity during the equinoctial seasons (Tretiach, 1993), particularly when

Species	Spain	Italy	Greece	Ticino	Israel
	ref	ref	ref	ref	ref
Abias caphalonica			7.11		
Aples cepitalonica Acor platanoidos			7,11	Q	
		1		9	
Allenthue altiesime	0	5.0		9	
Ananinus anissina Arbutus upada	9	J, Z		9	
Albuius uneuo	9			0	
Allius Ilicalia Potulo pondulo				9	
Corpinuo botuluo		1		9	
Carpinus beluius	0	1		9	
Corrilus spp.	9	1		9	
Corylus aveilaria	9	I		9	
Crataegus spp.		0		9	
Fagus sylvalica		2		9	
Frangula alhus		0.0		9	
Fraxinus exceisior	0	6; 2		9	
Fraxinus ornus	9	1			
Juglans regia	9			0	
Morus spp.				9	
Myrtus communis	9				_
Pinus halepensis	4	10	4; 12		8
Pinus pinea	_				8
Pistacia lentiscus	9				
Pistacia terebintus	9				
<i>Populus</i> spp.	9	3		9	
Prunus amygdalus	9				
Prunus avium		6; 2		9	
Prunus serotina				9	
Prunus spinosa	9	1			
Robinia pseudoacacia		2			
Rhamnus spp.				9	
<i>Rosa</i> spp.	9	1		9	
Salix spp.		1		9	
Sambucus spp.	9			9	
<i>Tilia</i> spp.		1		9	
Ulmus glabra		2		9	
Viburnum spp.	9	1		9	
Reference			7 Heliotic o	at al. 1988	
1 Bussotti (pers comm.)			8 Naveh et	al 1980	
2 Cozzi et al 2000			9 Skollvot	al 1999	
$2.  \bigcup Z = [a], Z = [a], Z = [a]$			10 Soda of	al., 1000	
A Gimono et al. 1909			11 Voliocoria	al., 2000	
Gravano at al 1000			12 Volioporia	n and oniello, 1999 Ni otal 1000	
5. Gravano et al., 1999;				יט כו מו., וששב	
o. Gravano et al., 2000a					

Table 2. Some of the most important native (or naturalized) tree and shrub species that showed ozone-like symptoms in Mediterranean countries.

Table 3. Sensitivity to ai	r pollution of mediterranean fore	st species, and relevant experimental cond	litions in chronological order (modified an	id updated from Bussotti and Ferretti, 1998)
Species Citrus limon	Reference Grimaldi e Polizzi, 1989	Kind of experiment Plants in fumigation chambers	Treatment Ozone: 0.6 – 0.7 ppm per 24 h.	Results Necrotic patches and ultrastructural alterations of mesophyll.
Pinus halepensis	Gimeno <i>et al.</i> , 1992	Field observations and controlled environment chambers (5 years-old seedlins)	Ozone both in field (up to 210 ppb) and in controlled conditions (70 ppb average for 7 h at day within 2 months)	Visible symptoms (chlorotic mottles)
Pinus halepensis	Wellburn and Wellburn, 1994	2-year-old seedlings in fumigation chambers	Episodic (up to 5 d) ozone exposure (up to 120 ppb) during a growing season	Visible symptoms (chlorotic mottles); starch accumulation in the endodermis; crushing of the phloem sieve cells.
Pinus halepensis	Anttonen <i>et al.</i> , 1994	18 month-old seedlings in controlled-environmental chambers	Ozone (150-600 ppb) over a period of 2-16 days	Low ozone exposure: increase in myristic and palmitic acid; reduction in chloroplast size and darkening of stroma;High ozone exposure: reduction of linolenic acid; disruption of chloroplast membranes.
Pinus halepensis	Manes and Blasi, 1995	4 year-old seedlings in fumigation chambers	Ozone: 150 ppb per 10 weeks, per 6 days week <sup>-1</sup> , per 7 h d <sup>-1</sup>	Reduction of photosynthetic activity.
Pinus halepensis	Elvira <i>et al.</i> , 1995	1-3 year-old seedlings in open top chambers	Ozone (filtered air; not filtered; ambient air + 40 ppb ozone). Summer treatments (7 h at day, 5 d at week) over a three year period	Visible symptoms in the second year of exposure. Reduction in net photosynthesis, stomatal conductance, chlorophyll, N and P.
Pinus halepensis	Peñuelas <i>et al.</i> , 1995	1-3 year-old seedlings in open top chambers	Ozone (filtered air; not filtered; ambient air + 40 ppb ozone). Summer treatments (7 h at day, 5 d at week) over a three year period	Decrease of chlorophyll content in the needles during the summer.
Pinus halepensis	Scalet <i>et al.</i> , 1995	3 year-old seedlings in growth chambers	Ozone (100 ppb) and simulated acid rain (pH 3.4) over a 2 months period	Increase of peroxidase activity, polyamine, putrescine and spermidine in 1-year needles both in combined and ozone treatments

Decrease in specific activity of whole plant. Increase of mitochondrial activity. With drought stress Rubisco activity decreases.	No significant effects of single treatments on root and total biomass. In combination O <sub>3</sub> and SO <sub>2</sub> reduce of 25% the total biomass and affect the mycorrhizae.	Depression of nitrate reductase; accumulation of polyamines, glutathione and ascorbate in current year needles. Drought and air pollution reduce total phenols and glutathione.	Increase in diffusive stomatal conductance. No significant decrease in starch content. Visible symptoms. Decrease in starch content. Increase in diffusive stomatal conductance.	Antagonistic effects on gas exchange rates between ozone and drought treatments.	Increase in extracellular and total peroxidase activities and in zeaxanthin levels in NFA+40 treatment.	Photosynthesis, chlorophyll fluorescence, POD activities are not influenced in treatments lower than 300 ppb	Ozone diminishes the growth both of seedling and fungus. CO <sub>2</sub> does not ameliorate the negative effects
Ozone (100 ppb) 14 h d <sup>-1</sup> during 3 months	Ozone (50 ppb) and SO <sub>2</sub> (40 ppb) single and together over 1 year.	Episodic ozone (100-110 ppb) fumigations	Ozone (1.2-1.8 x ambient concentration) over 1-2 growth seasons.Ozone (150 ppb) for 5 weeks (12 h d)	Ozone (filtered air; ambient air + 40 ppb ozone). Summer treatments (7 h at day, 5 d at week) over a 20 months period.Drought treatments.	Ozone: charcoal-filtered air (CFA); non-filtered air (NFA); non-filtered air + 40 ozone 40 ppb(NFA+40). Three consecutive summers (9 h at day, 5 d at week).	Ozone (0, 65, 175, 300 ppb) per 4 days, 6 h d <sup>-1</sup>	Ozone 200 ppb + CO <sub>2</sub> 700 µm for 68 days
3 year-old seedlings in growth chambers	2 year-old seedlings in fumigation chambers	3-year-old seedlings in solardomes	18 month-old seedlings in open air experiment.3 years-old seedlings in environmental growth chamber.	2 years-old seedlings in open top chambers	1 year-old seedlings in OTC	4 year-old seedlings in growth chambers	9 week-old seedlings (in symbiosis with <i>Paxillus involutus</i> ) in growth chambers
Gerant <i>et al.</i> , 1996	Diaz <i>et al.</i> , 1996	Wellburn <i>et al.</i> , 1996	Anttonen <i>et al.</i> , 1998	Inclán <i>et al.</i> , 1998	Elvira et al., 1998	Manes <i>et al.</i> , 1998	Kytoviita et al., 1999
Pinus halepensis	Pinus halepensis	Pinus halepensis	Pinus halepensis	Pinus halepensis	Pinus halepensis	Quercus ilex	Pinus halepensis

or on af	edles.	o. <i>ballota</i> tion of <i>allota</i> and	and ssynthetic	ir ir	nthetic of tomatal ;; nce; icrease of	١٤	) ppb; mis and pb.
No effects on CO <sub>2</sub> assimilation stomatal conductance. Reducti Rubisco activity.	Chlorotic mottling, reduction of chlorophyll and carotenoid concentration in C and C+1 ne	Visible symptoms on <i>Q. ilex</i> sst and <i>A. unedo</i> (NFA+40). Reduc plant biomass on <i>Q. ilex</i> ssp. <i>b</i> <i>O. europaea</i> ssp. sylvestris.	Visible symptoms on <i>A. unedo</i> <i>N. oleander.</i> Reduction of phot activity in all species.	Visible symptoms on all the spe investigated, in filtered and in a	Frantoio: reductions in photosy activity (57%) and stomatal conductance (69%); decrease stomatal opening, increase of s density Moraiolo: necrotic spot decrease of stomatal opening, ir stomatal density	Visible symptoms; ultrastructura alterations in the mesophyll	Visible symptoms at 50 and 100 structural changes in the epide walls more pronounced at 100.
Ozone 200 ppb over three months	Charcoal filtered air (CFA); non filtered air (NFA); non filtered air + ozone 50 ppb (NFA+O <sub>3</sub> ); ambient air (AA), 24 h d <sup>-1</sup> during a growth season.	Charcoal filtered air (CFA); non filtered air (NFA); non filtered air + ozone 40 ppb (NFA+40), 9 h d <sup>-1</sup> (10:00-18:00) during 1 year.	Ozone, single treatment at 200 ppb per 5 hours.	Charcoal filtered air (50% ambient); non filtered air (96% ambient); open air, during a growth season	Ozone (<3 and 100 ppb) per 120 days, 5 h d¹	AOT40 20 ppm.h during a growth season with hourly peaks over 100 ppb.	Ozone exposure at 0, 50 and 100 ppb for 21 days, 5 h d <sup>-1</sup>
3 year-old seedlings in phytotrone	2 year-old seedlings in OTC	2 year-old seedlings in OTC	1 year-old plants in fumigation chamber	Seedlings in OTC	5 year-old olive plants ( <i>Olea europaea</i> L. cvs. Frantoio and Moraiolo) in fumigation chamber	3 year-old potted seedlings in open air	2 years-old seedlings in fumigation chambers
Fontaine et al., 1999	Manninen et al., 1999	Inclán <i>et al.</i> , 1999	Lorenzini et al., 1999	Skelly et al., 1999	Minnocci et al., 1999	Soda et al., 2000	Bussotti et al., 2002
Pinus halepensis	Pinus halepensis	Quercus ilex ssp ilex; Q. ilex ssp. ballota; Olea europaea ssp. sylvestris; Ceratonia siliqua; Arbutus unedo	Arbutus unedo; Hedera helix; Laurus nobilis; Nerium oleander; Viburnum tinus	Arbutus unedo; Myrtus communis; Pistacia lentiscus; Pistacia terebintus	Olea europaea	Pinus halepensis	Arbutus unedo

30

the new leaves sprout. So, high spring ozone levels may be more dangerous than the summer ones. Furthermore, because of the emission of isoprene and monoterpenes, background summer levels of ozone are naturally higher in Mediterranean ecosystems than in other environments, so that plants are perhaps normally adapted to moderate levels of this pollutant. Yet, ozone-induced symptoms have been observed, although sporadically, in various Mediterranean forest species, suggesting that the influence of certain environmental factors can modify the situation and increase the plants' sensitivity to this pollutant. Visible symptoms and physiological disorders have been reproduced in Mediterranean species in experimental exposure conducted in controlled and semi-controlled environments. Worthy of note are the Mediterranean-mountain populations, that is the southern provenances of forest species very common throughout Europe. The behaviour towards ozone of some of these species (e.g. beech and silver fir) has been investigated on a European scale, however the findings of these studies cannot be extrapolated to southern populations because of the genetic, morphological and ecological differences. The data available appear to suggest that these provenances have a greater resistance to ozone.

The sensitivity of Mediterranean plant species to ozone varies considerably and is dependent on intra- and infra-specific differences as well as on ontogenetic factors, on the local availability of water and other factors such as soil fertility. The combination of these factors can modify plants' biological response. For example, in natural conditions of water shortage, even sensitive species such as *Pinus halepensis* do not appear to be particularly threatened (Barnes *et al.*, 1999).

In order to ensure the practical applicability of environmental protection policies, it is extremely important to establish communication networks between environmental protection and economic interest protection sectors (Innes and Price, 1999; Winter, 1999). In the Mediterranean region economic interests are hardly ever related to timber production; it would be more realistic in this region to envision a

### References

- Acherar, M., Rambal, S. & Lepart, J. 1991. Evolution du potential hydrique foliaire et de la conductance stomatique des chênes méditerranéens lors d'une period de dessêchement. Annales de Sciences Forestières 48:561-573.
- Alper-Siman, T., Peleg, M., Matveev, V., Mahrer, Y., Seter, I. & Luria, M. 1997. Recirculation of polluted air masses over the East Mediterranean coast. Atmospheric Environment 31:1441-1448.

cooperation with sectors involved in land conservation and tourism. For example, an important aspect of the photochemical smog-induced damage is the air's loss of transparency (Copeland, 1999), which clearly jeopardizes enjoyment of the landscape. Furthermore, one should not neglect the impact of ozone on human health. One should make it clearer that protecting the health of ecosystems and protecting the health of mankind are two aspects of the same problem, and can be addressed by the same policies.

To conclude, we feel that it would be useful to list briefly the gaps in our current scientific knowledge, which make difficult the establishment of a serious environmental protection policy against ozone in Mediterranean ecosystems:

- in the Mediterranean region, ozone climatology is still to a large extent unknown; equally, little is known of ozone exposure except in summer, or of the role of biogenic emissions in determining the background levels of this pollutant;
- direct effects of ozone on Mediterranean plant species are still to a large extent unknown, since there is a lack of specific and realistic cause-effect experiments;
- the sensitivity of southern provenances (Mediterranean-montane) of species common throughout Europe (e.g. beech, silver fir) still needs to be investigated;
- the impact of ozone on typically Mediterranean plant communities, such as coppices, is totally unknown;
- it is necessary to identify the most sensitive sectors within each ecosystem (tree renewal, meadows, herbaceous species etc.), i.e. those that can be used as "response indicators".

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- Anttonen, S., Herranen, J., Peura, P. & Kärenlampi, L. 1994. Fatty acids and ultrastructure of ozone-exposed Aleppo pine (*Pinus halepensis* Mill.) needles. Environmental Pollution 87:235-242.
- Anttonen, S., Kittilä, M. & Kärenlampi, L. 1998. Impacts of ozone on Aleppo pine needles: visible symptoms, starch concentration and stomatal responses. Chemosphere 36:663-668.
- Bacci, P., Sandroni, S. & Ventura, A. 1990. Patterns of tropospheric ozone in the pre-alpine region. Science of Total Environment 96:297-312.

Journal of Mediterranean Ecology vol.3, No 2-3 2002

- Barnes, J.D., Gimeno, B.S., Davison, A.W., Bussotti, F., Velissariou, D. & Gerant, D. 1999. Air pollution impacts on Mediterranean pine forests. pp.1-14. In: Ne'eman, G., Traband L. (eds.), Ecology, biogeography and management of *Pinus halepensis* and *Pinus brutia* forest ecosystems in the Mediterranean basin. Backhuys, Leiden.
- Bauer, G., Schulze, E.D. & Mund, M. 1997. Nutrient contents and concentrations in relation to growth of *Picea abies* and *Fagus sylvatica* along a European transect. Tree Physiology 17:777-786.
- Benton, J., Fuhrer, J., Gimeno, B.S., Skärby, L., Palmer-Brown, D., Ball, G., Roadknight, C. & Mills, G. 2000. An international cooperative programme indicates the widespread occurrence of ozone injury on crops. Agriculture Ecosystems Environment 78:19-30.
- Bussotti, F. & Ferretti, M. 1998. Air pollution, forest condition and forest decline in southern Europe. An overview. Environmental Pollution 101:49-65.
- Bussotti, F., Gravano, E., Grossoni, P., Tani, C. & Mori B. 2002. Ultrastructural responses of a Mediterranean evergreen shrub (*Arbutus unedo* L.) fumigated with ozone (in press) In: Karnosky, D.F. Percy, K.E., Chappelka, A.H., Simpson, C.J. (eds.), Air Pollution, Global Change and Forests in the New Millennium. Elsevier Science Ltd., Oxford, U.K.
- Butkovic, V., Cvitas, T. & Klasing, L. 1990. Photochemical ozone in the Mediterranean. Science of Total Environment 99:145-151.
- Chaloulakou, A., Assimacopoulos, D. & Lekkas, T. 1999. Forecasting daily maximum ozone concentrations in the Athens basin. Environmental Monitoring Assessment 56:97-112.
- Chappelka, A.H. & Samuelson, L.J. 1998. Ambient ozone effects on forest trees of the eastern United States: a review. New Phytologist 139:91-108.
- Copeland, S. 1999. Visibility impairment in the San Bernardino Valley. pp.106-125. In: Miller, P.R., McBride, J.M. (eds.),Oxidant air pollution impacts in the mountain forests of Southern California, Ecological Studies 134, Springer, New York.
- Cozzi, A., Ferretti, M. & Innes, J.L. 2000. Sintomi fogliari attribuibili ad ozono sulla vegetazione spontanea in Valtellina. Monti e Boschi 3-4:42-49.
- Davies, T.D. & Schuepbach, E. 1994. Episodes of high ozone concentrations at the earth's surface resulting from transport down from the upper troposphere/lower stratosphere: a review and case studies. Atmospheric Environment 28:53-68.
- Davison, A.W. & Barnes J.D. 1998. Effects of ozone on wild plants. New Phytologist 139:135-151.
- De Lillis, M. 1991. An ecomorphological study of the evergreen leaf. Braun-Blanquetia 7:1-126.
- Diaz, G., Barrantes, O., Honrubia, M. & Gracia, C. 1996. Effect of ozone and sulphur dioxide on mycorrhyzae of *Pinus halepensis* Mill. Annales de Sciences Forestières 53:849-856.
- Elvira, S., Alonso, R., Inclan, R., Bermejo, V., Castillo, F. & Gimeno, B.S. 1995. Ozone effects on Aleppo pine seedlings (*Pinus halepensis* Mill.) grown in open top chamber. Water Air and Soil Pollution 85:1387-1392.
- Elvira, S., Alonso, R., Castillo, F.J. & Gimeno, B.S. 1998. On the response of pigments and antioxidants of *Pinus*

*halepensis* seedlings to Mediterranean climatic factors and long-term ozone exposure. New Phytologist 138:419-432.

- Emberson, L.D., Ashmore, M.R., Cambridge, H.M., Simpson, D. & Tuovinen, J.P. 2000. Modelling stomatal ozone flux across Europe. Environmental Pollution 109:403-413.
- Evans, L.S. & Miller, P.R. 1972. Comparative needle anatomy and relative ozone sensitivity of four pine species. Canadian Journal of Botany 50:1067-1071.
- Fehsenfeld, F., Calvert, J., Fall, R., Goldan, P., Guenther, A.B., Hewitt, C.N., Lamb, B., Liu, S., Trainer, M., Westberg, H. & Zimmerman, P. 1992. Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. Global Biogeochemical Cycles 6:389-430.
- Flagler R.B. (ed.) 1998. Recognition of air pollution injury to vegetation: a pictorial atlas. Air & Waste Management Association. Pittsburgh, Pennsylvania, USA.
- Fontaine, V., Pelloux, J., Podor, M., Afif, D., Gerant, D., Grieu, P. & Dizengremel, P. 1999. Carbon fixation in *Pinus halepensis* submitted to ozone. Opposite response of ribulose – 1,5 – biphosphate carboxylase/ oxygenase and phosphoenolpyruvate carboxylase. Physiologia Plantarum 105:187-192.
- Fortezza, F., Strocchi, V., Giovannelli, G., Bonasoni, P. & Georgiadis, T. 1993. Transport of photochemical oxidants along the north-western Adriatic coast, Atmospheric Environment 27A:2393-2402.
- Fruekilde, P., Hjorth, L., Jensen, N.R., Kotzias, D. & Larsen, B. 1998. Ozonolysis at vegetation surface: a source of acetone, 4-oxopentanal, 6 –methyl-5-hepten-2-one, and geranyl acetone in the troposphere. Atmosferic Environment 32:1893-1902.
- Fumagalli, I., Matteucci, G., Schenone, G., Botteschi, G. & Buffoni, A. 1989. Effetti dell'inquinamento atmosferico sul pioppo ibrido in un sito rurale padano. Cellulosa e Carta 3:7-12.
- Georgiadis, T., Giovanelli, G. & Fortezza, F. 1994. Vertical layering of photochemical ozone during land-sea breeze transport. Il Nuovo Cimento 17C:371-375.
- Gerant, D., Podor, M., Grieu, P., Afif, D., Cornu, S., Morabito, D., Banvoy, J., Robin, C. & Dizengremel, P. 1996. Carbon metabolism enzyme activities and carbon partitioning in *Pinus halepensis* Mill. exposed to mild drought and ozone. Journal of Plant Physiology 148:142-147.
- Gerosa, G., Spinazzi, F. & Ballarin-Denti, A. 1999. Tropospheric ozone in Alpine Forest sites: air quality monitoring and statistical data analysis. Water Air and Soil Pollution 116:345-350.
- Gimeno, B.S., Velissariou, D., Barnes, J.D., Inclán, R., Peña, J.M. & Davison, A. 1992. Daños visibles por ozono en aciculas de *Pinus halepensis* Mill. en Grecia y España. Ecologia 6:131-134.
- Gravano, E., Ferretti, M., Bussotti, F. & Grossoni, P. 1999. Foliar symptoms and growth reduction of *Ailanthus altissima* in an area with high ozone and acidic deposition in Italy. Water Air and Soil Pollution 116:267-272.
- Gravano, E., Bussotti, F., Grossoni, P. & Tani, C. 2000a. Danni fogliari da ozono: caratterizzazione ultrastrutturale di *Fraxinus excelsior* L. e *Prunus avium* L. pp.447-452 In: Bucci, G., Minotta. G., Borghetti, M. (eds.), Ap

plicazioni e prospettive per la ricerca forestale italiana. SISEF Atti 2, Edizione Avenue Media, Bologna, Italy.

- Gravano, E., Desotgiu, R., Tani, C., Bussotti, F. & Grossoni, P. 2000b. Structural adaptations in leaves of two Mediterranean evergreen shrubs under different climatic conditions. Journal of Mediterranean Ecology 1:165-170.
- Grimaldi, V. & Polizzi, G. 1989. Ultrastruttura di foglie di limone dopo esposizione a dosi elevate di ozono. Tecnica Agricola 41:143-154.
- Gucci, R., Massai, R., Casano, S. & Mazzoleni, S. 1999. Seasonal changes in the water relations of Mediterranean co-occurring woody species. Plant Biosystems 133:117-128.
- Güsten, H., Heinrich, G., Cvitas, T., Klasing, L., Ruscic, B., Lalas, D.P. & Petrakis, M. 1988. Photochemical formation and transport of ozone in Athens, Greece. Atmospheric Environment 22:1855-1861.
- Gutschick, V.P. 1999. Biotic and abiotic consequences of differences in leaf structure. New Phytologist 144:3-18.
- Heath, R. 1999. Biochemical processes in an ecosystem: how should they be measured? Water Air and Soil Pollution 116:279-298.
- Heliotis, F.D., Karandinos, M.G. & Whiton, J.C. 1988. Air pollution and the decline of the fir forest in Parnis National Pak, near Athens, Greece. Environmental Pollution 54:29-40.
- Hjellbrekke, A.G. 2000. Ozone measurements 1998. Kjeller, Norwegian Institute for Air Research (EMEP/CCC-Report 3/98 O-7727), Norway.
- Inclan, R., Alonso, R., Pujadas, M., Terés, J. & Gimeno, B.S. 1998. Ozone and drought stress: interactive effects on gas exchange in Aleppo pine (*Pinus halepensis* Mill.). Chemosphere 36:685-690.
- Inclán, R., Ribas, A., Peñuelas, J. & Gimeno, B.S. 1999. 'The relative sensitivity of different Mediterranean plant species to ozone exposure. Water Air and Soil Pollution 116:273-277.
- Innes J.L. & Price, C. 1999. Determination of economic losses associated with ozone impacts on European forests. pp.121-124. In: Fuhrer, J., Achermann, B. (eds.), Critical levels for ozone – Level II. Environmental Documentation No. 115. Swiss Agency for the Environment, Forests and Landscape (SAEFL), CH.
- Innes, J.L., Skelly, J.M. & Schaub, M. 2001. Ozone and broadleaved species: a guide to the identification of ozoneinduced foliar injury. Haupt, Berne, CH.
- Kangasjärvi, J., Talvinen, J., Utiainen, M. & Karjalainen, R. 1994. Plant defence systems induced by ozone. Plant Cell and Environment 17:783-794
- Karabourniotis, G., Kyparissis, A. & Manetas, Y. 1993. Leaf hairs of *Olea europaea* protect underlying tissues against ultraviolet-B radiation damage. Environmental and Experimental Botany 33:341-345.
- Kärenlampi, L. & Skärbi, L., 1996. 'Critical levels for Ozone in Europe. Testing and finalizing the concepts. UN-ECE Workshop report, published by the University of Kuopio, Finland, Dept. Ecology and Environmental Science.
- Kolb, T.E., Fredericksen, T.S., Steiner, K.C. & Skelly, J.M. 1998. 'Issues in scaling tree size and age response to ozone: a review. Environmental Pollution 98:195-208
- Kytoviita, M.M., Pelloux, J., Fontaine, V., Botton, B. & Dizengremel, P. 1999. Elevated CO, does not ameliorate

effects of ozone on carbon allocation in *Pinus halepensis* and *Betula pendula* in symbiosis with *Paxillus involutus*. Physiologia Plantarum 106:370-377.

- Lalas, D.P., Asimakopulos, D.N., Deligiorgi, D.G. & Helmis, C.G. 1983. Sea breeze circulation ad photochemical pollution in Athens, Greece. Atmospheric Environment 17:1621-1632.
- Larsen, J.B. 1990. Effects of ozone on gas exchange, frost resistance, flushing and growth of different provenances of European silver fir (*Abies alba* Mill.). European Journal of Forest Pathology 20:211-218.
- Lorenzini, G., Nali, C. & Biagioni, M. 1995. Long range transport of photochemical ozone over the Tyrrhenian Sea demonstrated by a new miniaturized bioassay with ozone-sensitive tobacco seedlings. Science of the Total Environment 166:193-199.
- Lorenzini, G., Nali, C., Ligasacchi, G. & Ambrogi, R. 1999. Effects of ozone on photosynthesis of Mediterranean urban ornamental plants. Acta Horticulturae 496:335-338.
- Loreto, F., Förster, A., Dürr, M., Csiky, O. & Seufert, G. 1998. On the monoterpene emission under heat stress and on the increased thermotolerance of leaves of *Quercus ilex* L. fumigated with selected monoterpenes. Plant, Cell and Environment 21:101-107.
- Maier-Maercker, U. 1998. Predisposition of trees to drought stress by ozone. Tree Physiology 19:71-78.
- Manes, F. & Blasi, C. 1995. Environmental stress and Mediterranean vegetation. Fresienus Environmental Bullettin 4:183-188.
- Manes, F., Vitale, M., Giannini, M. & Paoletti E. 1998.  $O_3$ and  $O_3+CO_2$  effects on a Mediterranean evergreen broadleaf tree, holm oak (*Quercus ilex* L.). Chemosphere 36:801-806.
- Manninen, S., Le Thiec, D., Nourisson, G., Radnai, F., Garrec, J.P. & Huttunen, S. 1999. Pigment concentrations and ratios of Aleppo pine seedlings exposed to ozone. Water Air and Soil Pollution 116:333-338.
- Martín, M., Plaza, J., Andrés, M.D., Bezares, J.C. & Millám, M.M. 1991. Comparative study of seasonal air pollutant behaviour in a Mediterranean coastal site: Castellón (Spain). Atmospheric Environment 25:1523-1535.
- Millán, M., Artiñano, B., Alonso, L., Navazo, M. & Castro, M. 1991. The effect of meso-scale flows on the regional and long range atmospheric transport in the Western Mediterranean area. Atmospheric Environment 25A:949-963.
- Millán, M., Salvador, R., Mantilla E. & Artiñano, B. 1996. Meteorology and photochemical air pollution in Southern Europe: experimental results from EC research projects. Atmospheric Environment 12:1909-1924.
- Miller, P.R. & McBride, J. (eds) 1999. Oxidant air pollution impacts in the mountain forests of Southern California. Ecological Studies 134. Springer Verlag, Berlin.
- Miller, P.R., Parmeter, J.R. jr, Taylor, O.C. & Cardiff, E.A. 1963. Ozone injury to the foliage of *Pinus ponderosa*. Phytopathology 53:1072-1076.
- Minnocci, A., Panicucci, A., Sebastiani, L., Lorenzini, G. & Vitagliano, C. 1999. Physiological and morphological responses of olive plants to ozone exposure during a growing season. Tree Physiology 19:391-397.
- Naveh, Z. 1995. Conservation, restoration, and research priority for Mediterranean uplands threatened by global climate changes. pp. 482-507. In: Moreno, J.M., Oechel,

W.C. (eds.), Global change and Mediterranean-type ecosystems. Ecological Studies 117. Springer Verlag. Berlin, Germany.

- Naveh, Z., Steinberger, E.H., Chaim, S. & Rotmann, A. 1980. Photochemical air pollutants. A threat to Mediterranean coniferous forest and upland ecosystems. Environmental Conservation 7:301-309.
- Paludan-Müller, G., Saxe, H. & Leverenz, J.W. 1999. Response to ozone in 12 provenances of European beech (*Fagus sylvatica*): genotypic variation and chamber effects on photosynthesis and dry matter partitioning. New Phytologist 144:261-273.
- Peñuelas, J. & Lusià, J. 1999. Short-term responses of terpene emission rates to experimental changes of PFD in *Pinus halepensis* and *Quercus ilex* in summer field conditions. Environmental Experimental Botany 42:61-68.
- Peñuelas, J., Filella, I., Elvira, S. & Inclan, R. 1995. Reflectance assessment of summer ozone fumigated Mediterranean white pine seedlings. Environmental and Experimental Botany 35:299-307.
- Polle, A. & Rennenberg, H. 1992. Field studies on Norway spruce trees at high altitudes. II. Defence systems against oxidative stress in needles. New Phytologist 121:635-642.
- Posch, M., Hetteling, J.P., De Smet, P.A.M. & Downing, R.J. (eds.) 1998. Calculation and mapping of critical thresholds in Europe: CCE Status report 1997. RIVM Rep. 259101007, Bilthoven, Netherlands.
- Rhizopoulou, S. & Mitrakos, K. 1990. Water relations of evergreen sclerophylls. I. Seasonal changes in the water relations of eleven species from the same environment. Annals of Botany 65:171-178
- Rinallo, C. & Gellini, R. 1989. Morphological and anatomical traits identifying the silver fir (*Abies alba* Mill.) from the Serra S. Bruno Provenance. Giornale Botanico Italiano 122:149-166.
- Salleo, S. & Lo Gullo, M.A. 1990. Sclerophylly and plant water relations in three Mediterranean *Quercus* species. Annals of Botany 65:259-270.
- Sanz, M.J. & Millán, M.M. 1998. The dynamics of polluted air masses and ozone cycles in the western Mediterranean: relevance of forest ecosystems. Chemosphere 36:1089-1094.
- Sanz, M.J. & Millán, M.M. 2000. Ozone in the Mediterranean region: evidence of injury to vegetation. pp.165-192. 'In: Innes, J.L. (ed.). 'Forest dynamics in heavily polluted regions. IUFRO Research Series 1, CABI Publishing, Wallingford, UK.
- Sanz, M.J. & Calatayud, V., Calvo, E. 2000. Spatial pattern of ozone injury in Aleppo pine related to air pollution dynamics in a coastal-mountain region of eastern Spain. Environmental Pollution 198:239-247.
- Scalet, M., Federico, R., Guido, M.C. & Manes, F. 1995. Peroxidase activity and polyamine changes in response to ozone and simulated acid rain in Aleppo pine needles. Environmental and Experimental Botany 35:417-425.
- Schlager, H., Graf, J., Krautstrunk, M. & Brünner, M. 1992. Final MEMOSA Project Report, D.L.R., Institut für Physik der Atmosphäre, Oberpfaffenhofen (D).
- Skärbi, L., Ro-Poulsen, H., Wellburn, F.A.M. & Sheppard, L.J. 1998. Impacts of ozone on forests: a European perspective. New Phytologist 139:109-122.

- Skelly, J.M., Davis, D.D., Merrill, W., Cameron, E.A., Brown, H.D., Drummond, D.B. & Dochinger, L.S. 1987. Diagnosing injury to eastern forest trees. University Park, Penn State University, College of Agriculture. Pennsylvania.
- Skelly, J.M., Innes, J.L., Snyder, K.R., Savage, J.E., Hug, C., Landolt, W. & Bleuler, P. 1998. Investigations of ozone-induced injury in forests of Southern Switzerland: field surveys and open-top chamber experiments. Chemosphere 36:995-1000.
- Skelly, J.M., Innes, J.L., Savage, J.E., Snyder, K.R., Vanderheyden, D., Zhang, J. & Sanz, M.J. 1999. Observation and confirmation of foliar ozone symptoms of native plant species of Switzerland and Southern Spain. Water Air and Soil Pollution 116:227-234.
- Soda, C., Bussotti, F., Grossoni, P., Barnes, J.D., Mori, B. & Tani, C. 2000. Impacts of urban levels of ozone on *Pinus halepensis* Mill. foliage. Environmental and Experimental Botany 44:69-82.
- Street, R., Owen, S., Duckham, S., Boissard, C. & Hewitt, C.N. 1997. Effect of habitat and age on variations in emission from *Quercus ilex* and *Pinus pinea*. Atmospheric Environment 31:89-100.
- Tattini, M., Gravano, E., Pinelli, P., Mulinacci, N. & Romani, A. 2000. Flavonoid secreted by glandular trichomes play a key role in the acclimatation mechanism of *Phillyrea latifolia* L. to high solar radiation. New Phytologist 112:215-220.
- Tretiach, M. 1993. Photosynthesis and transpiration of evergreen Mediterranean and deciduous trees in an ecotone during a growing season. Acta Oecologica 14:341-360
- VanderHeyden, D.J., Skelly, J.M., Innes, J.L., Hug, C., Zhang, J., Landolt, W. & Bleuler, P. 2001. Ozone exposure thresholds and foliar injury on forest plants in Switzerland. Environmental Pollution 111:321-331.
- Velissariou, D. & Skretis, L. 1999. Critical levels exceedances and ozone biomonitoring in the greek fir forest (*Abies cephalonica* Loud.) at the Parnis mountain national park in Attica, Greece. pp.205-208. In: Fuhrer, J., Achermann, B. (Eds.), Critical levels for ozone Level II. Environmental Documentation No. 115. Swiss Agency for the Environment, Forests and Landscape (SAEFL), CH.
- Velissariou, D., Davison, A.W., Barnes, J.D., Pfirmann, T., Mac Lean, D.C. & Holevas, C.D. 1992. Effects of air pollution on *Pinus halepensis* Mill.: pollution levels in Attica, Greece. Atmospheric Environment 26:373-380.
- Wellburn, F.A.M. & Wellburn, A.R. 1994. Atmospheric ozone affects carbohydrate allocation and winter hardiness of *Pinus halepensis* Mill. Journal of Experimental Botany 45:607-614.
- Wellburn, F.A.M., Lau, K.K., Milling, P.M.K. & Wellburn, A.R. 1996. Drought and air pollution affect nitrogen cycling and free radical scavenging in *Pinus halepensis* Mill. Journal of Experimental Botany 47:1361-1367.
- Winter, P.L. 1999. Human aspects of air quality in the San Bernardino Mountains. In: Miller, P.R., McBride, J.R. (Eds.). Oxidant air pollution impacts in the mountain forests of Southern California. A case study of the San Bernardino Mountains. Ecological Studies 134. Springer-Verlag, Berlin, pp.373-393.